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TITLE: Method for determining the structure of oligosaccharide linkages and automated instrumentation thereof

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INVENTOR-INFORMATION:

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US-CL-CURRENT: 436/94, 422/70 , 422/82.05 , 422/82.09 , 435/4 , 436/171 , 436/173

ABSTRACT:

The invention provides a method for determining the structure of a carbohydrate sample, comprising perbenzoylating a carbohydrate sample with a perbenzoylating agent to protect free hydroxyl groups in the carbohydrate sample; cleaving the glycosidic linkages of the perbenzoylated carbohydrate sample by contacting the carbohydrate sample with an amount of BrCH.sub.2 COBr/H.sub.2 O effective to cleave the carbohydrate sample; treating the resulting product with AgOAc and methanol or AgOTf/TMU and methanol to effect glycosidation; treating the resulting product with thiourea to remove bromoacetate groups; subjecting the resulting product to effect methoxycinnamoylation of free hydroxyl groups; separating the resulting benzoates with high-pressure liquid chromatography; performing mass, ultraviolet and circular dichroic spectroscopy on the separated benzoates; and comparing the spectra so obtained with reference spectra or calculated values to identify the structure of the carbohydrate. The invention also provides an apparatus for automatically determining the structure of a carbohydrate molecule such as an oligosaccharide.

18 Claims, 136 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 136

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Brief Summary Text - BSTX (5):

Neither of these instruments is applicable to structural or analytical work involving sugars. Increasing interest in the structure and properties of such compounds has created a demand for sophisticated yet simplified analysis of oligosaccharides and other types of sugars. Unfortunately, oligosaccharide structure is more difficult to determine because it involves ascertaining the structure of the saccharide subunits and their absolute configuration, and finally their anomeric structure.

Detailed Description Text - DETX (25):

Determination of linkage structure in oligosaccharides has long relied upon methylation analysis by GLC/MS of partially methylated alditol acetates (3). These monosaccharide residues are obtained by hydrolysis of permethylated oligosacchrides, reduction of the anomeric center, and acetylation of the remaining hydroxyls which were originally involved in linkages. Methylation analysis generally requires a minimum of 25 nmol of material, although capillary GLC with smaller quantities have been reported (4). The analysis relies heavily on chromatographic separation and comparison of derivatized monosaccharide GLC retention times with a large bank of synthetic standards, few of which are commercially available. Linkage analysis generally follows a